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A Survey of Microbiological Quality of Fish Sold in Two Local Wet Markets

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ABSTRAK

Lapan spesies ikan laut diperolehi dari pasar-pasar berdekatan (Sungai Besi dan Kajang). Ikan-ikan telah dianalisis bagi jumlah kiraan hidup, koliform, Vibrio parahaemolyticus *dan bakteria penghasil-histamina. Jumlah kiraan hidup diperolehi menggunakan Agar 'Plate Count'; koliform menggunakan kaldu McConkey;* Vibrio parahaemolyticus *dengan agar TCBS dan bakteria penghasil-histamina menggunakan media Niven. Jumlah kiraan bakteria pada kesemua ikan dari kedua-dua pasar adalah di antara julat 10^6 hingga 10^8 per gram ikan, koliform di antara 10^2 hingga 10^5 per gram, dan semua ikan yang dikaji adalah positif bagi kedua-dua* Vibrio parahaemolyticus *dan bakteria penghasil-histamaina.*

ABSTRACT

Eight marine fish species were obtained from two nearby wet markets (Sungei Besi and Kajang). The fish were analysed for total viable counts, coliforms, Vibrio parahaemolyticus *and histamine-producing bacteria. Total viable count was determined using Plate Count Agar; coliforms using McConkey Broth;* Vibrio parahaemolyticus *by TCBS agar and histamine-producing bacteria were determined using Niven's media. The total counts on all fish from both markets ranged from 10^6 to 10^8 per gram of fish, coliforms ranged from 10^2 to 10^5 per gram, and all the fish examined were positive for both* Vibrio parahaemolyticus *and histamine-producing bacteria.*

INTRODUCTION

In Malaysia, fish are sold in local wet markets at ambient temperatures. Fish are transported in lorries over long distances in either iced or frozen conditions.

In Kuala Lumpur, fish are brought to a central location by wholesalers who then sell to retailers who come from surrounding towns or communities within a 45 km radius. The wholesalers begin to sell fish at about 3 a.m. and by 6 a.m. the retailers have bought the fish and are on their way to their respective destinations. Fish are rarely iced either at the wholesale market or during transportation by the retailers.

The flesh of freshly caught healthy fish is generally sterile but the skin and gills and slime may carry high loads of bacteria (Shewan 1961; Simmonds and Lamprecht 1985). The spoilage of fish is primarily due to bacterial activity, although endogenous proteolytic and autolytic enzymes do play an important role. The spoilage of fish is normally caused by psychrophillic and psychrotrophic microorganisms (Shewan 1977). Psychrotrophic microorganisms can grow at temperatures between 0–7° and have an optimum growth temperature of 15-20°C (Jay 1986).

According to the 1973 FAO Code of Practice for Fresh Fish, fish should be handled at all times with great care and in such a way as to inhibit the growth of microorganisms. Fish quality deteriorates rapidly and potential keeping time is shortened if they are not handled and stored properly.

Microbiological surveillance of seafoods often includes analysis for indicator microoganisms such as coliforms, faecal coliforms and

enterococci whose presence indicates poor sanitation and the possible presence of enteric pathogens.

Vibrio parahaemolyticus, a food poisoning organism, is widely distributed in nature and has been isolated from coastal waters worldwide (Blake 1980). The organism is normally present in estuarine environments and not in the open sea (Colwell 1984). It is cold-sensitive and does not survive in seawater below 10°C (Thomson and Thacker 1973). *V. parahaemolyticus* also is known to be lethally and sublethally injured during refrigeration or frozen storage.

Histamine poisoning is a chemical intoxication that results from ingestion of food containing unusually high levels of histamine. Histamine is formed by bacteria which possess the enzyme histidine decarboxylase. Bacteria that are frequently responsible for histamine poisoning in fish are *Morganella morganii, Klebsiella pneumoniae* and perhaps other enteric bacteria (Taylor 1986).

The objective of this study was to determine the microbiological quality of fish obtained from 2 local markets. The microbiological quality was assessed by determining the aerobic plate count, coliforms, *V. parahaemolyticus* and histamine-producing bacteria.

MATERIALS AND METHODS

Eight species of fish were bought in the early morning from fishmongers at Sungei Besi and Kajang markets, then brought back to the laboratory and analysed immediately. The skin layer from 5 fishes of the same species was removed and pooled, and 1 gm of the skin was analysed for aerobic plate count, coliforms, *V. parahaemolyticus* and histamine-producing bacteria.

Aerobic plate count (APC) was determined by the spread-plate method, using triplicate plates per dilution with Plate Count Agar (Oxoid). Plates with 25-250 colonies were selected to determine the APC. Coliforms were determined using the 3-tube Most Probable Number (MPN) method using McConkey Broth (Oxoid).

V. parahaemolyticus was enumerated by the spread plate method using TCBS agar (Oxoid) in triplicates per dilution. Colonies which were colourless and with green centres were recorded as presumptive *V. parahaemolyticus*.

Histamine-producing bacteria were isolated using Niven's differential medium (Niven *et al.* 1981). Isolates possessing histidine decarboxylase activity having a halo zone around the colony were considered as positive.

RESULTS AND DISCUSSION

Results of aerobic plate counts and coliforms for all fish examined are given in Table 1.

APC results were in the range of 10^6 to 10^7 except for *Megalaspis cordyla* from Kajang which had an APC of 8.3×10^5/g. *Rastrelliger sp., Selaroides leptolepsis* and *Selar crumenopthalmus* from Sungei Besi market had APC of 1.04×10^7, 2.16×10^7 and 1.72×10^7, respectively, while the same fish from Kajang market had APC of 2.8×10^6, 1.95×10^6 and 9.6×10^6, respectively, which were one log cycle less than those obtained from Sungei Besi market. *Nem-*

TABLE 1
Aerobic Plate Count (APC) and Coliforms on different species of fish obtained from Sungai Besi and Kajang markets per g of fish

TYPE OF FISH	SG. BESI		KAJANG	
	APC	Coliform	APC	Coliform
Rastrelliger sp.	1×10^7	2.53×10^5	2.8×10^6	2.4×10^4
Selaroides leptolepsis	2.16×10^7	2.4×10^5	1.95×10^6	2.4×10^5
Nemipterus sp.	8.6×10^6	2.4×10^3	1.85×10^7	2.4×10^6
Scomberomorus sp.	1.96×10^6	9.5×10^2	1.39×10^7	4.6×10^4
Megalaspis cordyla	1.05×10^6	3.5×10^4	8.3×10^5	4.3×10^4
Decapterus sp.	1.35×10^6	3.5×10^4	1.05×10^7	4.6×10^5
Parastromatens niger	1.46×10^6	1.56×10^5	1.41×10^6	1.1×10^6
Selar crumenopthalmus	1.72×10^7	2.4×10^5	9.6×10^6	1.1×10^6

ipterus sp., *Scomberomorus* sp., *Megalaspis cordyla* and *Decapterus* sp. from Sungei Besi market had APC of 8.6×10^6, 1.96×10^6, 1.05×10^6 and 1.35×10^6, respectively whereas *Nemipterus* sp., *Scomberomorus* sp. and *Decapterus* sp. from Kajang market had APC of 1.85×10^7, 1.39×10^7 and 1.03×10^7, respectively.

Results showed high coliform counts, ranging from 10^2 to 10^6. *Rastrelliger* sp., *Selaroides leptolepsis, Nemipterus* sp., *Parastromatens niger* and *Selar crumenopthalmus* obtained from Sungei Besi market had coliform counts in the range of 1.56 to 2.5×10^5 while *Megalaspis cordyla* and *Scomberomorus* sp. had 3.5×10^4 coliforms/g of fish. *Scomberomorus* sp. from the same market had the lowest coliform count of 9.5×10^2. *Nemipterus* sp., *Parastromatens niger* and *Selar crumenopthalmus* from Kajang market had coliform counts in the range of 1.1 to 2.4×10^6/g while *Selaroides leptolepsis, Scomberomorus* sp. *Decapterus* sp. and *Sardinella* sp. had coliform counts in the range of 2.4 to 4.6×10^5. *Rastrelliger* sp. and *Megalaspis cordyla* had coliform counts in the range of 2.4 to 4.3×10^4. There was not much difference in APC and coliform counts of fish obtained from Sungei Besi and Kajang market as the difference was about one log cycle.

Malaysian Food Regulation, 1985, stipulates that fish meant for consumption should not have APC exceedig 5×10^5 and coliforms 5×10 per gram of fish. This study shows that APC count for all the fish, except for *Megalaspis cordyla*, exceeded by one to two log cycles. Coliforms exceeded by five to six log cycles. Presence of coliforms indicate harvesting of fish from contaminated environments, poor sanitation, contamination during processing and temperature abuse during storage and transportation. Presence of coliforms can also indicate the presence of pathogens. The FAO (1973) report recommended that fish should be cooled down to the temperature of melting ice, 0°C, as quickly as possible. Barile *et al.* (1985) found that the shelf-life of Faughn's Mackerel on ice was reduced by one day for each hour delay in icing or exposure to ambient temperatures of 28-30°C. The storage life decreases with increasing storage temperature; for example, Cape Hake which kept for 9 days in ice, lasted for 5, $2^1/2$ and $1^1/2$ days respectively at 5, 10 and 15°C (Simmonds and Lamprecht 1985).

Results in Table 2 show the presence of different species of *Vibrio* that are capable of growing on TCBS. The counts range from 7×10 to 9.4×10^2/g of fish, obtained from Sungei Besi market, while the counts for fish obtained from Kajang ranged from 2.8×10^2 to 2.12×10^5. Table 2 also shows the percentage of isolates that have similar morphological characteristics to *V. parahaemolyticus*. Most of the fish that were obtained from Sungei Besi and Kajang markets had high percentage of presumptive *V. parahaemolyticus*. *Megalaspis cordyla* from both Kajang and Sungei Besi markets had a lower per cent of presumptive *V. parahaemolyticus*. *V. parahaemolyticus* was found to

TABLE 2

Presumptive counts of *Vibrio parahaemolyticus* and other *Vibrio* spp on different species of fish obtained from Sungei Besi and Kajang markets per g of fish

TYPE OF FISH	SG. BESI			KAJANG MARKET		
	Total No. of organisms on TCBS Agar	% Yellow colonies	% Green colonies	Total No. of organisms on TCBS Agar	% Yellow colonies	% Green colonies
Rastrelliger sp.	3.5×10^2	0.00	100.00	2.8×10^2	53.37	46.43
Selaroides leptolepsis	9.4×10^2	10.64	89.36	4.0×10^2	25.00	75.00
Nemipterus sp.	4.7×10^2	2.13	97.87	2.12×10^5	26.70	73.30
Scomberomorus sp.	4.8×10^2	73.96	26.04	3.7×10^2	54.80	45.20
Megalaspis cordyla	7.0×10^1	28.57	71.43	3.7×10^2	78.40	21.60
Decapterus sp.	3.0×10^2	18.64	81.36	5.7×10^2	34.51	65.49
Parastromatens niger	4.8×10^2	14.74	85.26	4.7×10^2	6.45	93.55
Selar crumenopthalmus	7.0×10^2	12.95	87.05	3.9×10^2	0.00	100.00

TABLE 3
Percentage of histamine-producing bacteria on Niven's media isolated from different species of fish obtained from Sungei Besi and Kajang markets per g of fish

TYPE OF FISH	SG. BESI		KAJANG MARKET	
	Total No. of organisms on Niven's medium	% Histamine producing bacteria	Total No. of organisms on Niven's medium	% Histamine producing bacteria
Rastrelliger sp.	1.47×10^5	13.52	2.21×10^4	9.34
Selaroides leptolepsis	5.9×10^3	22.45	8.1×10^4	15.80
Nemipterus sp.	1.34×10^5	7.66	2.6×10^2	0.0
Scomberomorus sp.	2.75×10^5	3.98	3.7×10^2	35.14
Megalaspis cordyla	2.77×10^3	16.86	4.4×10^2	11.50
Decapterus sp.	2.49×10^5	12.95	6.7×10^5	1.97
Parastromatens niger	2.43×10^5	19.65	1.31×10^5	8.40
Selar crumenopthalmus	2.48×10^5	18.15	2.34×10^5	3.85

be a common contaminant on fish obtained from both markets. As *V. parahaemolyticus* is sensitive to cold, the presence of *V. parahaemolyticus* suggests that the fish was not kept at low ambient temperature during storage and transportation. Failure to maintain low temperature favoured the growth of these microorganisms.

Results in Table 3 show the presence of histamine-producing bacteria on Niven's medium. All the fish obtained from both markets, except for *Nemipterus* sp. from Kajang contained histamine-producing bacteria. Unlike presumptive *V. parahaemolyticus*, the percentage of histamine-producing microorganisms was low. According to Taylor 1986, histamine-producing bacteria are usually considered to be part of the normal microflora of the gut, skin or gills of fish. Growth and histamine production can be prevented by keeping the fish at low temperature.

Results of this study show that fish obtained from both Sungei Besi and Kajang markets have high aerobic plate counts and coliform counts; and are positive for both presumptive *V. parahaemolyticus* and histamine-producing bacteria. As fish are sold at ambient temperatures, the presence of these microorganisms do pose a health hazard. Presence of coliforms indicates possible faecal contamination and hence the possibility of the presence of pathogens such as *Salmonella* or other pathogenic enteric viruses. Keeping fish at ambient temperature till the end of the business day will favour the growth of *V. parahaemolyticus*, which is known to cause gastroenteritis, and histamine-producing bacteria which are present in low numbers.

If fish is to be sold at ambient temperatures, it is strongly recommended that facilities for the provision of cheap ice be made available and fish be sufficiently iced at all markets. This will ensure that fish sold to consumers will be of adequate quality.

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